Integration of multiple immune-associated biomarkers facilitates classification of solid tumors by primary immune escape mode and prediction of patient outcomes

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Introduction

- Many individual biomarkers describe the complexity of each tumor and its interactions with the tumor microenvironment (TME).
- However, tumors often evade immunotherapy through multiple immune escape mechanisms.
- Here, we present a method of integrating immune and neoplastic biomarkers that classify tumor and immune activity in the TME.

Methods

- Standard-of-care comprehensive genomic and immune profiling was performed on 5450 FFPE tumors representing 39 histologic types, assessing expression levels of 395 immune genes and >500 tumor-associated genes [1,2].
- From this data, three previously published gene expression signatures were calculated: cell proliferation (CP), tumor immunogenic signature (TIGS), and cancer testis antigen burden (CTAB) [3,4,5].
- PD-L1 status of each tumor was assessed by IHC, and tumor mutational burden (TMB) was calculated.
- The five key variable distributions were centered about their means and scaled by their standard deviations.
- Principle component analysis (PCA) and unsupervised clustering of the resulting five-dimensional data revealed four distinct biological groups, here termed “phenotypes”.
- Expressing each data point and the centroids of the four phenotypes in terms of the five principle components calculated above, a nearest centroid method was used to classify a validation cohort of 110 immune checkpoint inhibitor (ICI) treated non-small cell lung cancer (NSCLC) patients into these phenotypes.
- The association between these phenotypes and ICI treatment response in the NSCLC cohort was assessed by calculating the disease control rate (DCR) as the fraction of total patients observed to have complete response (CR), partial response (PR), or stable disease (SD) according to RECIST.
- Overall survival of the phenotypes in the NSCLC cohort was assessed using Kaplan-Meyer and CoxPH analyses.

Results

PCA and clustering generated four groups: 1) Tumor Dominant, exhibiting high CTAB, TMB, and CP, and low PD-L1 and TIGS; 2) Proliferative, exhibiting high CP and low TIGS, PD-L1, CTAB, and TMB; 3) Inflamed, exhibiting high TIGS and low CP, PD-L1, CTAB, and TMB; and 4) Checkpoint, exhibiting high PD-L1, TIGS, and TMB, and low CTAB (Fig. 1).

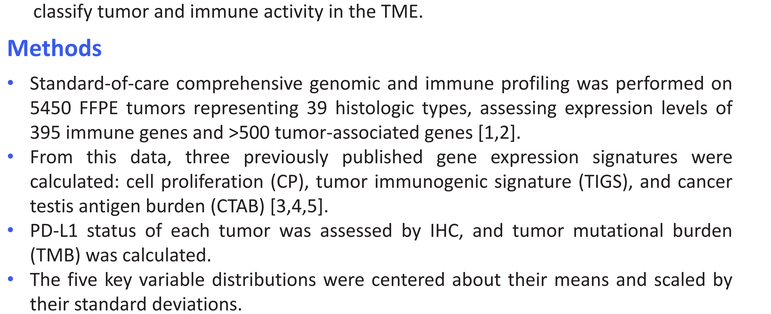

Figure 1: PCA and hierarchical clustering results: A) eigenvector plot detailing the influence of the five constituent biomarkers on the first two principle components, B) classification of the 110 patient NSCLC validation cohort into the four phenotypes, C) distribution of the four phenotypes within the validation cohort.

Kaplan-Meier survival analysis suggested a significant relationship between these phenotypes and overall survival [p=0.022], with the checkpoint phenotype demonstrating increased survival over all others and phenotype stratification demonstrating greater increase in median survival than stratification by any constituent biomarker (Fig. 2).

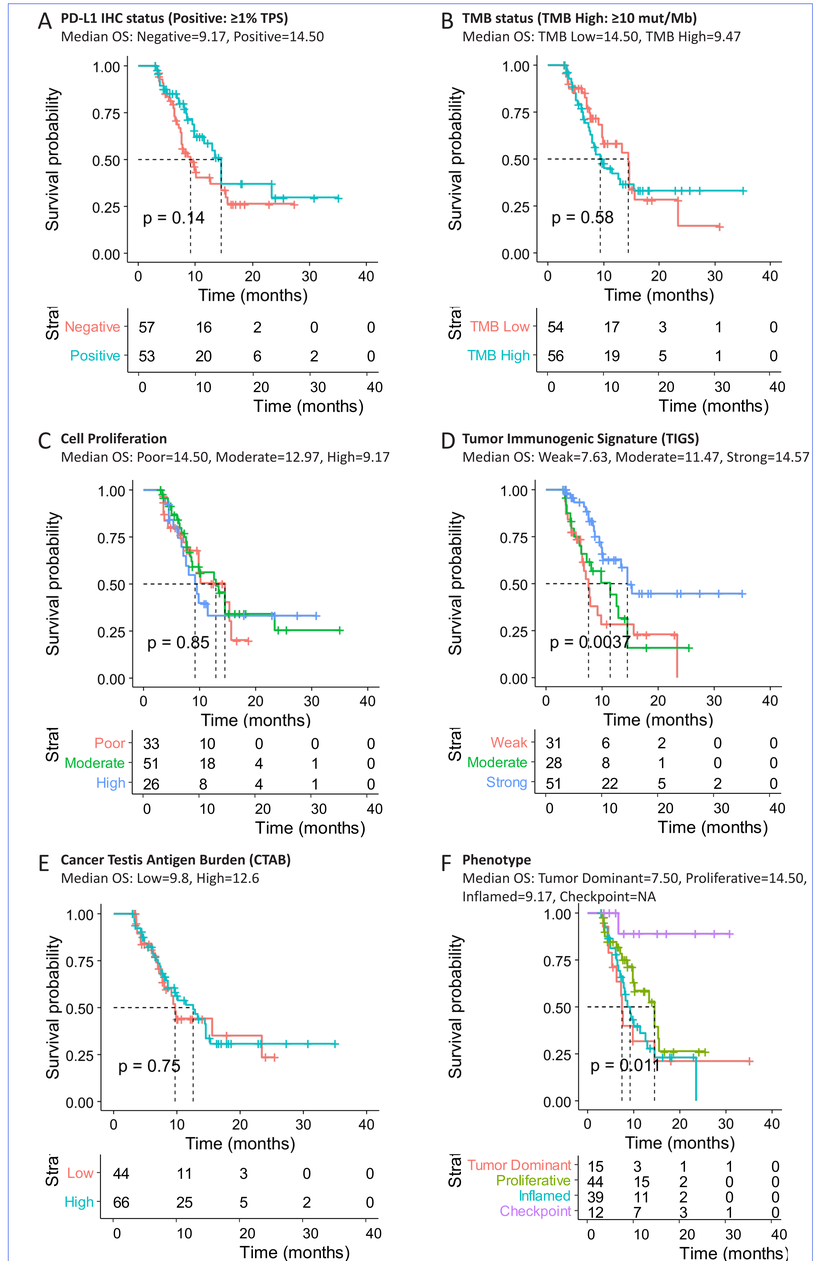

Figure 2: Kaplan-Meier survival analyses for NSCLC validation cohort (n=110) as stratified by: A) PD-L1 IHC status, B) TMB status, C) cell proliferation, D) tumor immunogenic signature (TIGS) , E) cancer testis antigen burden (CTAB), F) phenotype.

CoxPH analysis showed the checkpoint group to have a significantly decreased hazard ratio [HR=0.10; p=0.038] for ICI treatment (Fig. 3). As in Kaplan-Meier analysis, this approach outperformed any of its constituent biomarkers as a survival predictor in CoxPH analysis.

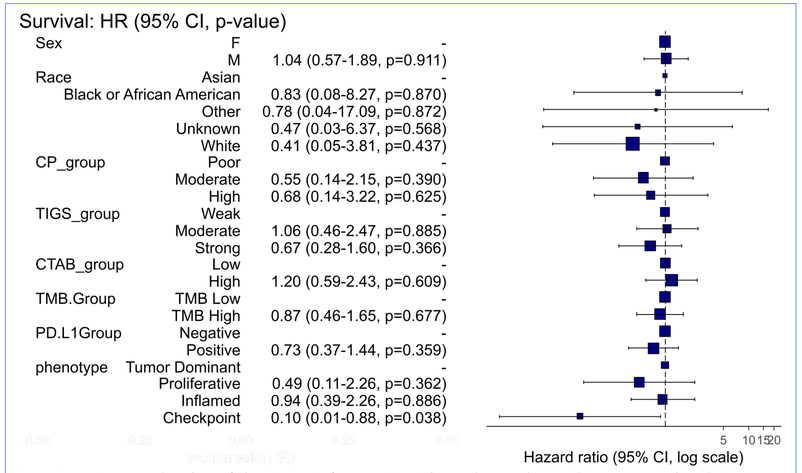

Figure 3: CoxPH survival analysis of phenotype, its five constituent biomarkers, and sex and race as survival predictors.

Calculating the DCR for each of the four phenotypes, the checkpoint phenotype was found to have the highest DCR [0.417] (Fig. 4). This value was greater than both the next highest phenotype DCR [0.273] and the cohort average [0.236].

Figure 4: Disease control rate (DCR) for the 110 NSCLC patient validation cohort as subdivided into the four phenotypes: tumor dominant [DCR=0.200], proliferative [DCR = 0.273], inflamed [DCR=0.154], and checkpoint [DCR=0.417].

Conclusions

- An integrated approach combining comprehensive tumor profiling and emerging biomarkers outperforms single IO markers to predict ICI response and survival.
- Checkpoint phenotype had best survival predictive capability.
- Divergent outcomes between the resulting groups are likely the result of distinct tumor-immune interaction modalities.
- As we further validate this methodology, we hope to produce a treatment decision and clinical trial selection strategy that leverages standard of care comprehensive genomic and immune profiling in solid tumors to outperform single marker testing.

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